

Prolonged Hyporesponsiveness of Airway Smooth Muscle to Histamine Following General Anesthesia

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The prolonged effect of barbiturates and inhalation anesthesia on airway response to histamine was studied in five groups of dogs. Group B (n = 10), H24 (n = 5), and H72 (n = 5) were anesthetized with sodium thiamylal (B) or halothane and N₂O (H24, H72) for 3 h, during which a dose-response curve to histamine was obtained. The animals were then allowed to recover; 24 h (B and H24) or 72 h later, the animals were killed and an *in vitro* dose-response curve to histamine was obtained on a tracheal smooth muscle (TSM) specimen. Group C (n = 5), the control group, had no prolonged exposure to anesthesia. The dogs were immediately killed and the *in vitro* histamine response of the TSM was measured. The results (mean ± SE) showed that the smooth muscle contractile properties (*i.e.*, the maximum contraction to electrical field stimulation) were comparable in all four groups: 111 ± 12 g (B); 168 ± 23 g (H24); 106 ± 32 g (H72); and 107 ± 31 g (C). The maximum response (mean ± SE) to histamine (as % of maximum electrical contraction) was: 15 ± 6% (B), 30 ± 9% (H24), 32 ± 12% (H72), and 50 ± 8% (C). Statistical analysis of the data showed that the histamine response of Group B and Group H24 was significantly decreased compared to Group C ($P < 0.01$ and < 0.05 , respectively); in Group H72 the results were not significantly different from Group C ($0.1 > P > 0.05$). To exclude the possibility that the diminution in histamine response was due to the *in vivo* exposure to histamine, a fifth group of animals served as sham. The animals were anesthetized with sodium thiamylal and killed 24 h later, having inhaled no histamine. The results obtained in this group were comparable with those obtained in Group B. The authors conclude that exposure to general anesthetics decreased airway smooth muscle sensitivity to histamine for at least 24 h after the cessation of anesthesia. (Key words: Airway: resistance. Anesthetics, intravenous: thiamylal. Anesthetics, volatile: halothane. Histamine: airway response. Muscle, smooth: airway).

IT IS KNOWN THAT airway sensitivity to irritants is decreased in the presence of general anesthetics, including barbiturates and volatile agents. This has been shown *in vivo* in dogs and guinea pigs¹⁻³ and *in vitro* on guinea pig and ferret tracheal chain preparations.⁴⁻⁶ It is generally assumed that this effect is reversible and that the airway

smooth muscle regains its sensitivity shortly after the cessation of anesthesia. The purpose of this study was to determine whether exposure to general anesthesia may in fact have a long-term effect. This question is of interest to both the clinician and the scientist. For the clinician, the knowledge that airway sensitivity to irritants, and therefore the likelihood of developing an asthma attack, is increased, decreased, or unchanged following general anesthesia could affect the postoperative management of asthmatic patients. From a research point of view, it is important to know whether studies on airway sensitivity conducted on airway specimens taken from an individual or an animal under general anesthesia might be influenced by the use of anesthetic agents.

The effects of two different types of general anesthesia on airway smooth muscle sensitivity to histamine were examined. The first was anesthesia with a short-acting barbiturate (sodium thiamylal), which was chosen because it is often used by respiratory physiologists in animal experimentation. The other type of anesthesia was a mixture of halothane and nitrous oxide, which are anesthetics frequently used in human surgery, particularly in asthmatic patients.

Materials and Methods

PROTOCOL

Five groups of dogs were studied.

Group B. Ten dogs were anesthetized with sodium thiamylal and paralyzed with pancuronium for 2-3 h. During the anesthesia, a dose-response curve to histamine was obtained. Twenty-four hours postanesthesia, the animals were killed and tracheal smooth muscle (TSM) sensitivity to histamine was measured.

Group H24. Five dogs were anesthetized with halothane and nitrous oxide only and paralyzed with pancuronium for 2-3 h. A dose-response curve to histamine was obtained during anesthesia. The animals were killed 24 h later and TSM sensitivity was studied.

Group H72. Five dogs were given anesthesia and histamine exposure as in Group H24 but the TSM was studied 72 h postanesthesia.

Group C. Five dogs in whom only TSM sensitivity was studied. These animals had no prolonged anesthesia prior to the *in vitro* measurements.

Group S. Five dogs that followed the same protocol as Group B dogs with the exception that histamine dose-response curves were replaced by NaCl inhalations.

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IN VIVO EXPERIMENTS

Twenty mongrel dogs, weighing between 17 and 32 kg, were anesthetized either with intravenous sodium thiamylal, 18 mg/kg, or halothane and nitrous oxide. With the gaseous mixture, anesthesia was induced *via* mask using increasing concentrations of halothane and N₂O, thus completely avoiding the use of barbiturates. Anesthesia was then maintained with a mixture of 1% halothane and 60% N₂O.⁷ The animals were paralyzed with pancuronium bromide, .08 mg/kg, intubated with a no. 9 endotracheal tube and mechanically ventilated with a constant-volume ventilator (Harvard® apparatus). The femoral artery was catheterized to obtain arterial blood samples for the measurement of pH, P_{CO₂}, and P_{O₂} and to permit the continuous recording of blood pressure *via* an HP267BC pressure transducer. In Groups B and S, the anesthesia was maintained by an intravenous infusion of thiamylal sodium at a rate of 0.1 mg · kg⁻¹ · min⁻¹. The level of curarization was regularly checked with a block aid monitor (Dupaco 54120®), and pancuronium was injected when needed *via* femoral vein. An esophageal balloon (5 cm × 1 cm) was positioned in the lower esophagus. Pressure at the airway opening was measured by a catheter with multiple side holes positioned 2–3 cm beyond the distal end of the endotracheal tube. Transpulmonary pressure (P_L) was obtained by subtracting esophageal pressure from airway opening pressure *via* a variable reluctance transducer (Validyne® MP ± 100 cm H₂O). Flow was measured with a heated Fleisch® pneumotachograph (Nr 3) coupled to a 270 Hewlett-Packard pressure transducer. The pneumotachograph was calibrated with the appropriate gas mixture: either air or the mixture of halothane, nitrous oxide, and oxygen. The animals lay in the prone position in a pressure-compensated (flow-integrated) body plethysmograph with a flat frequency response to 10 Hz. Pulmonary resistance (R_L) was determined on four consecutive breaths by the technique of Amdur and Mead. This method for measuring R_L consists of dividing the PL difference by the \dot{V} difference at points of equal volume during the respiratory cycle. All signals were recorded on a 7758A 8-channel, pen-heated, Hewlett-Packard® recorder.

Histamine Dose-Response Curves. A fresh histamine diphosphate solution, diluted with physiologic saline, was prepared prior to each experiment. The histamine solution was aerosolized *via* Devilbiss® no. 45 nebulizer connected to a no. 2a Rosenthal-French® dosimeter (Dr. R. S. Rosenthal, Johns Hopkins University).

Prior to the inhalation of histamine, the dog was insufflated three times to a P_L of 25 cm H₂O to ensure a constant volume history. It was then ventilated for 2 min, after which increasing doses of histamine were administered. The following doses were inhaled: 0.23, 0.46, 0.92,

1.84, 3.68, 7.36, 14.72, 29.44, and 55.88 mg. Doses were obtained by the following formula:

$$\text{ml aerosol generated} \times \text{number of breaths} \\ \times \text{drug concentration}$$

The experiment was terminated when a complete dose-response curve was obtained (*i.e.*, a plateau was reached) or when mean arterial blood pressure had dropped below 80 mmHg. Once the histamine dose-response curve was obtained, the animals were allowed to recover.

IN VITRO EXPERIMENTS

The animals were killed with an intravenous injection of sodium thiamylal immediately followed by an intravenous injection of saturated KCl.

A portion of extrathoracic trachea was dissected, and a specimen of TSM was prepared as described by Stephens.⁸ Two TSM strips were obtained from each dog. They were placed in 10 ml organ baths with a modified Krebs-Henseleit solution containing (mmol/l): Na⁺ 143.3; K⁺ 5.9; Ca²⁺ 2.6; Mg²⁺ 1.2; Cl⁻ 128.3; H₂PO₄⁻ 2.2; SO₄²⁻ 1.2; dextrose 10; EDTA 0.21; and HCO₃⁻ 24.9.

The solution was maintained at 37° C and gased with a mixture of 95% O₂ and 5% CO₂. The lower end of the specimen was attached to a fixed pin on the bottom of the bath *via* silk thread inserted through a small piece of cartilage kept at each end of the TSM. The upper end of the specimen was attached to a Grass FT.03® force transducer mounted on a rack-and-pinion adjustable clamp so that muscle length could be adjusted. Electrical field stimulation was provided by two platinum wire electrodes mounted parallel to the muscle strip and powered by a Grass S8® stimulator.

After 90 min incubation, the optimum length for tension generation (L_{max}) was defined as the length of smooth muscle at which the difference between active and passive tensions was maximal. It was determined by stretching the muscle by increments of 1 mm and measuring the passive and active tension (produced by an electrical stimulus of 45 V, 15 Hz, 5 ms pulse duration for 30 s).⁹ There was an interval of 10 min between each electrical stimulation. The muscle was held at L_{max} throughout the remainder of the experiment.

Fresh histamine diphosphate solutions were used for each experiment. The concentrations of the solutions were chosen so that the volume added to the bath never exceeded 100 μl. Noncumulative dose-response curves to histamine were obtained by allowing the TSM to return to its baseline level of tension after each successive dose of histamine. Because of the possibility of tachyphylaxis,¹⁰ only one dose-response curve was obtained on each strip. At the end of the experiment, the specimens were dis-

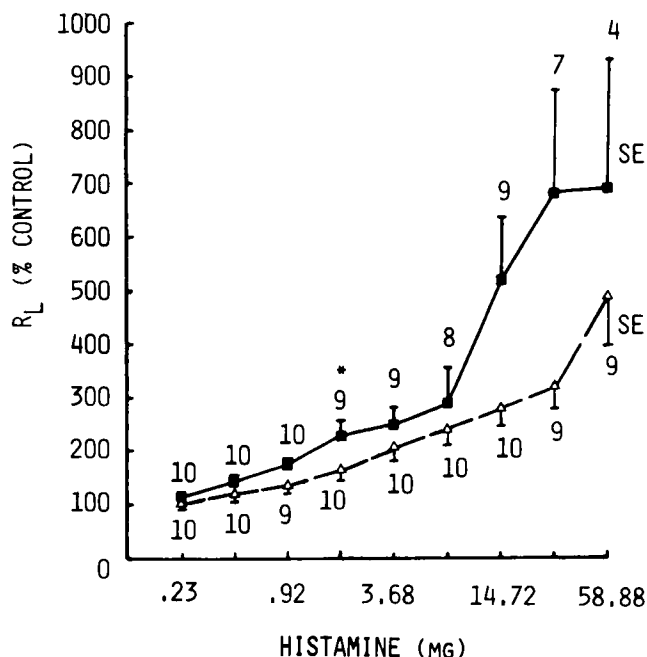


FIG. 1. *In vivo* dose-response curves to inhaled histamine during thiamylal (■—■) and halothane anesthesia (△---△) (mean ± SE). The numbers on each point represent n. Only at the dose of histamine, 1.84 mg, was the difference between the two groups significant ($P < 0.05$).

sected free of cartilage, blotted on a gauze pad, and weighed.

DATA ANALYSIS

In vivo Experiments. The data were analyzed with the two-tailed unpaired *t* test.

In vitro Experiments. The results were first analyzed with a two-way analysis of variance with unequal subclass number, followed by the Student-Newman-Keuls multiple comparison test. $P < 0.05$ was considered statistically significant.

Results

IN VIVO DATA

Prior to histamine inhalation, R_L was not significantly different during halothane- N_2O or thiamylal anesthesia: $1.7 \pm 0.6 \text{ cm H}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}^{-1}$ and $2.1 \pm 0.6 \text{ cm H}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}^{-1}$, respectively. Figure 1 shows that compared with thiamylal, halothane- N_2O anesthesia appears to protect the animals from histamine-induced bronchoconstriction. However, because of the wide interindividual variability in histamine responsiveness, the difference between the barbiturate anesthesia group and the halothane- N_2O group is statistically significant only at one dose of histamine. Arterial pressure (mean ± SE) was 151

$\pm 6 \text{ mmHg}$ (B), $131 \pm 5 \text{ mmHg}$ (S), $121 \pm 7 \text{ mmHg}$ (H24), $114 \pm 6 \text{ mmHg}$ (H72) during the control period and $65 \pm 5 \text{ mmHg}$ (B), $109 \pm 11 \text{ mmHg}$ (H24), and $79 \pm 11 \text{ mmHg}$ (H72) at the largest dose of histamine. In Group S (not exposed to histamine) mean arterial blood pressure was $141 \pm 7 \text{ mmHg}$ at the end of the experiment.

IN VITRO DATA

Table 1 shows the mean maximal tension developed with electrical stimulation. To normalize for differences in weights, the results are indicated in g tension/g weight. The data show that there was no difference in the maximal tension developed by the TSM specimens of animals exposed to halothane and nitrous oxide (Groups H24 and H72) or to sodium thiamylal (Groups B and S) and those of the control group (Group C).

Figure 2 shows the mean dose-response curves to histamine for the five groups. To normalize for differences in intrinsic contractile properties of the TSM, the amplitude of the contraction induced by histamine is expressed as a per cent of the maximum contraction elicited by electrical stimulation. Analysis of the data shows that the TSM responses to the three largest doses of histamine ($5 \times 10^{-5} \text{ M}$, 10^{-4} M , and $5 \times 10^{-4} \text{ M}$) are not significantly different, indicating that the curves have reached a plateau. In addition, the analysis showed that the mode of anesthesia affects histamine sensitivity of the TSM specimens. The Student-Newman-Keuls multiple comparison test was used to compare the effect of anesthetics on the plateau (constituted of the three largest doses of histamine) of curves B, S, and H24.¹¹ The results of this analysis (table 2) show that: 1) there is no difference between Group S (*i.e.*, animals anesthetized with barbiturates but not exposed to histamine *in vivo*) and Group B (animals anesthetized with barbiturates and exposed to histamine); 2) compared with the control Group C, Groups S, B, and H24 are significantly different ($P < 0.01$, < 0.01 , < 0.05 , respectively); and 3) Groups H24 and B are not significantly different ($P < 0.10$).

The observation that halothane had a significant effect on TSM sensitivity to histamine 24 h after cessation of its administration prompted us to study how long this effect of halothane would last, so we studied Group H72. The results obtained on Group H72 show a $P < 0.10$ compared with Group C, suggesting that histamine sensitivity of the TSM may not return completely to normal 72 h after halothane administration.

Discussion

IN VIVO DATA

Control values for R_L were similar for halothane and thiamylal anesthesia and are comparable with those ob-

TABLE 1. Maximal Tension Developed by Electrical Stimulation at L_{\max} (g tension/g weight)

Group C (n = 5)	Group H24 (n = 5)	Group H72 (n = 5)	Group B (n = 10)	Group S (n = 5)
106.9 ± 69.9	167.5 ± 52.3	106.3 ± 71.3	110.8 ± 37.9	111.4 ± 28.8

Results are mean ± SD. Numbers in the group are in parentheses.

L_{\max} = optimum length for tension generation.

tained by Hirshman *et al.*^{2,12} in the same experimental conditions. Furthermore, in comparison with barbiturate anesthesia, halothane-nitrous oxide anesthesia tended to protect the animals from histamine-induced bronchoconstriction. In this respect too, our results are comparable with those of Hirshman *et al.*,² who compared the effects of thiopental, halothane, and isoflurane anesthesia on antigen- and methacholine-induced bronchoconstriction. Hirshman *et al.* suggested that inhalational anesthetics prevent airway constriction through two mechanisms: *via* a depression of reflex pathways and *via* a direct action on airway smooth muscle. The recent work of Korenaga *et al.*¹³ confirms this hypothesis. These authors published a detailed study of the mechanism of action of halothane on the dog TSM and found that halothane diminishes the contraction evoked by membrane depolarization and also raises the threshold membrane depolarization required to produce a contraction. Halothane also inhibits the development of twitch tension induced by nerve stimulation by diminishing the release of acetylcholine from nerve endings. Barbiturate anesthesia, whether with thiopental or with thiamylal, is less effective in preventing airway constriction by antigen, methacholine, or histamine. The effects of barbiturates on dog TSM have been studied in detail by Holtzman *et al.*¹⁴ They showed that in a dose-dependent fashion, barbiturates inhibit first the central nervous system synapses, followed by the postsynaptic membrane in parasympathetic ganglia. In addition, other authors have shown that barbiturates also depress transmission in sympathetic ganglia.¹⁵ In the concentrations we used, however, barbiturates do not produce a parasympathetic blockade.^{2,16}

In conclusion, these *in vivo* data are consistent with those of other authors and show that halothane-nitrous oxide anesthesia is more effective than barbiturate anesthesia in protecting against histamine-induced bronchoconstriction.

IN VITRO DATA

Our results show that for 24 h postanesthesia, there is a significant decrease in the TSM sensitivity to histamine. This decrease in sensitivity cannot be attributed to perioperative factors such as histamine-induced arterial hypotension as the sham group (Group S) showed a similar if not greater decrease in histamine sensitivity despite a

mean arterial pressure maintained stable during the whole experiment. Moreover, the results obtained on Group S indicate that the diminished TSM sensitivity cannot be attributed to histamine tachyphylaxis. Furthermore, we do not believe that the method of killing could have influenced the results as all the animals were killed in the same way.

Several *in vitro* studies have shown that TSM sensitivity to irritants such as histamine or acetylcholine is depressed in the presence of volatile anesthetic agents such as halothane and diethyl ether or in presence of barbiturates such as sodium thiopental.^{4,5} To our knowledge, however, a residual effect of general anesthetics on the TSM sensitivity has never been reported. In a recent publication, Spiss *et al.*¹⁷ have shown that the vascular smooth muscle of rabbit is still hyporesponsive to norepinephrine 4 h posthalothane anesthesia, but their results are not exactly

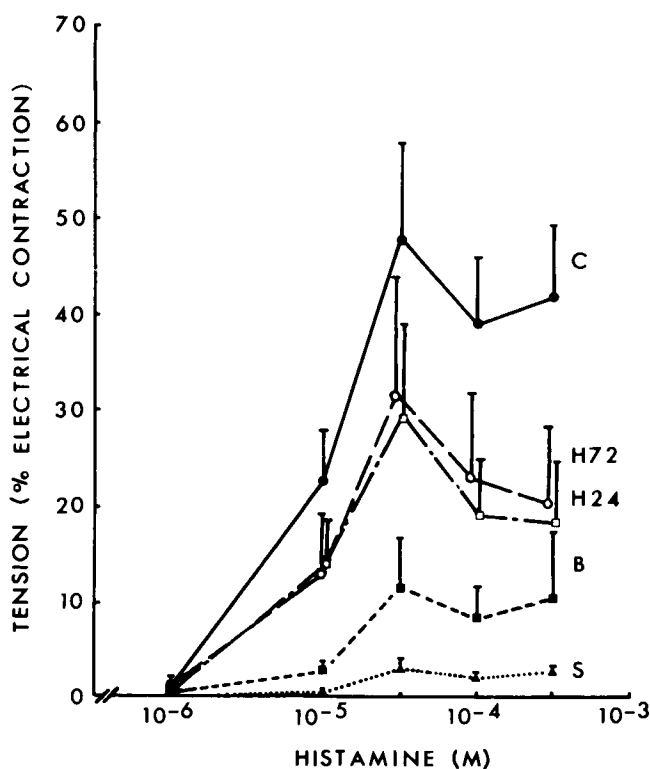


FIG. 2. *In vitro* dose-response curves to histamine (mean ± SE). C versus B = $P < 0.01$; C versus S = $P < 0.01$; C versus H24 = $P < 0.05$; C versus H72 = $0.1 > P > 0.05$; S versus B = NS.

TABLE 2. Results Obtained with the Student-Newman-Keuls Multiple Comparison Test

Group B	NS		
Group H24	$P < 0.10$	$P < 0.10$	
Group C	$P < 0.01$	$P < 0.01$	$P < 0.05$
	Group S	Group B	Group H24

NS = not significant.

comparable with ours as the animals were killed immediately following anesthesia. In one study, Skoogh *et al.*⁶ mention that 60 min after exposure to thiopental, the response to acetylcholine of a tracheal nerve preparation was still not back to control levels. These authors also mention that the barbiturate washout seemed slower in their tracheal nerve muscle preparation than in the tracheal ring preparation, and they attribute this slower washout to a greater amount of tissue left in the preparation. Indeed, it is well known that short-acting barbiturates are highly fat soluble and that the *in vivo* washout period can last up to a week.¹⁸ Thus, it is conceivable that barbiturate washout was not complete 24 h postanesthesia in our animals.

It is more difficult to understand how a gaseous mixture can influence smooth muscle response so long after its administration. A possible explanation lies in a recent publication of Wyrwicz *et al.*¹⁹ Using fluorine-19 nuclear magnetic resonance, the authors have found that residual fluorine-19 signals from halothane could still be detected in rabbit brains 98 h postanesthesia, despite the short duration of anesthesia (about 30 min). Considering that gas uptake by tissues is dependent on both solubility and time, it is quite conceivable that a significant amount of halothane was still present in our dogs 24 or even 72 h after an anesthesia of 3-h duration.

Based on the studies performed *in vitro* on the effects of halothane, we can then speculate that the postanesthetic decrease in airway sensitivity to histamine may be due to: 1) a residual inhibitory effect of halothane on the electrical and contractile properties of smooth muscle fibers;¹³ 2) a residual effect of anesthesia on synaptic transmission in autonomic ganglia.^{6,13,14} Indeed, although it is still a subject of debate, histamine probably does induce smooth muscle contraction by a dual mechanism, *i.e.*, by a direct action on smooth muscle, and by inducing the release of acetylcholine from nerve endings in the airway wall.⁹ If this latter action is still altered by anesthesia, histamine-induced constriction would be decreased. Finally, 3) halothane or thiamylal could also decrease the response to histamine by having a direct action on cellular receptors.

Considering that there was no difference between

groups in the maximal tension developed by electrical stimulation, it seems unlikely that our results can be explained by an effect of halothane on the neuroeffector transmission of the smooth muscle cells. Thus, it appears that anesthesia had a direct effect on the histamine receptors.

Besides halothane, two other factors, pancuronium and nitrous oxide, could have affected TSM sensitivity to histamine postanesthesia. Pancuronium is a nondepolarizing neuromuscular blocker with weak atropine-like activity and no direct effect on smooth muscle contractile properties. Nitrous oxide has a solubility coefficient much lower than that of halothane; thus, the washout period is considerably shorter. Nevertheless, until more complete, specific studies have been conducted, we cannot completely rule out a possible role of pancuronium or nitrous oxide in the postanesthetic decrease in TSM sensitivity to histamine.

Obviously, more detailed studies are necessary to understand the mechanisms of action of this residual effect of general anesthetics on TSM sensitivity to irritants. However, the fact that anesthesia influences smooth muscle response for so long after its cessation has important ramifications. For example, studies on TSM specimens obtained from surgery in humans or animals may have been affected by the type of anesthesia used. This is true particularly in regard to the recent studies attempting to correlate *in vivo* and *in vitro* smooth muscle sensitivity to histamine or methacholine, and their conclusions may thus be questioned.^{20,21} Our data also suggest that the beneficial bronchodilating effect of halothane observed during asthmatic attacks may be longer lasting than anticipated and that the routine postoperative administration of bronchodilators to asthmatic patients in remission may not be necessary.

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